Factors affecting toxicity of ferulate towards the cyanobacterium *Oscillatoria* cf *chalybea*

Kevin K Schrader,[1]* Agnes M Rimando,[1] Craig S Tucker[2] and Stephen O Duke[1†]

[1]*USDA, ARS, NPURU, School of Pharmacy, PO Box 8048, University of Mississippi, University, MS 38677, USA*
[2]*Delta Research and Extension Center, Mississippi State University, PO Box 197, Stoneville, MS 38776, USA*

Abstract: The objectives of this study were to determine the effect of ferulate and its analogs, and factors which may affect the toxicity of ferulate to *Oscillatoria* cf *chalybea*, the cyanobacterium presumed to cause musty flavor in farm-raised catfish. Rapid bioassays utilizing 96-well and six-well cell culture plates were used to monitor the toxicity of ferulate analogs and potential ferulate stability factors toward *O* cf *chalybea*. The additions of low concentrations of the oxidizing compound sodium carbonate peroxyhydrate with ferulate did not help control *O* cf. *chalybea*. Of three forms of ferulate tested, *trans*-ferulate was most toxic towards *O* cf *chalybea*. Light enhanced the toxicity of ferulate, indicating that weather conditions and the time of day could influence the success of ferulate applications to fish ponds to control *O* cf *chalybea*. Ferulate was less toxic to *O* cf *chalybea* in six-well culture plates than in 96-well plates, indicating that higher concentrations of ferulate should be used in field trials, due to possible reduction of ferulate toxicity towards *O* cf *chalybea* in large, aquatic environments (ie fish ponds). These studies provided fundamental information on potential ferulate toxicity towards *O* cf *chalybea* to be considered before conducting field trials (ferulate applications to fish ponds).
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1 INTRODUCTION

Undesirable flavor (off-flavor) in channel catfish (*Ictalurus punctatus* L) is responsible for large economic losses to catfish producers in Mississippi. Off-flavor catfish are rejected by processors and must be held in aquaculture ponds for additional periods of days or weeks until the fish lose their off-flavor and are determined to be of acceptable flavor quality. The most common off-flavor in channel catfish in west-central Mississippi is a musty taint caused by the accumulation of the compound 2-methylisoborneol (MIB) in the fish flesh.[1] The cyanobacterium *Oscillatoria* cf *chalybea* produces MIB in culture,[2] and *O* cf *chalybea* is presumed to be the major contributor of MIB in the water of catfish production ponds in west-central Mississippi.[3]

Methods for preventing off-flavor episodes in catfish production ponds include the application of algicides to the ponds. However, the only algicide currently approved by the United States Environmental Protection Agency for use in food-fish production ponds is copper sulfate, which is limited in its effectiveness in selectively controlling undesirable cyanobacteria (blue-green algae) due to its relatively broad-spectrum toxicity towards phytoplankton. The discovery of other compounds with greater specific toxicity towards noxious cyanobacteria would greatly benefit the channel catfish industry.

Recent studies have identified *trans*-ferulate [3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid] (Fig 1A) as selectively toxic to *O* cf *chalybea*.[4] *Trans*-ferulate was found to be inhibitory toward the growth of *O* cf *chalybea* at 1 μM, while 1000 μM of *trans*-ferulate was required to inhibit the growth of the green alga *Selenastrum capricornutum*.[4] At present, the mode of action of *trans*-ferulate on the growth of *O* cf *chalybea* is unknown.

Ferulate is a natural compound ubiquitous in plants and is probably released during the decomposition of plant material. Also, ferulate and some of its microbial derived metabolites are moderately phytotoxic to vascular plants.[5,6] In barley, ferulate is the major low molecular weight phenolic compound,[7] and recent laboratory studies found that decomposing barley straw had an algistatic effect on the growth of the cyanobacterium *Microcystis aeruginosa* Kuetz.[8] More recently, decomposing barley straw has been identified as a method for controlling the growth of certain species of cyanobacteria in water reservoirs.[9,10] Toxic oxidized phenolic compounds have been proposed as

* Correspondence to: KK Schrader, USDA, ARS, NPURU, School of Pharmacy, PO Box 8048, University of Mississippi, University, MS 38667, USA
†Previous address: USDA, ARS, SWSLU, Stoneville, MS 38776, USA
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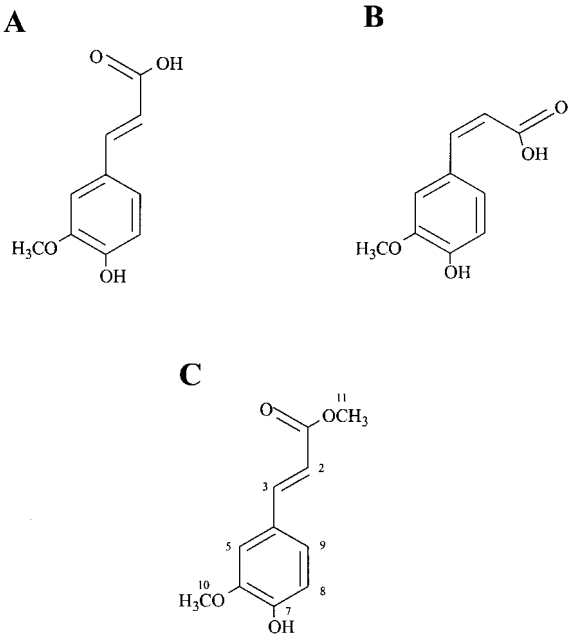

Figure 1. Chemical structures of (A) *trans*-ferulate, (B) *cis*-ferulate, and (C) *trans*-methylferulate.

the algicidal principles produced during the decomposition of barley straw.[11] The phenolic compound tannic acid may also be released during barley straw decomposition and can undergo auto-oxidation when aerated and at alkaline pH.[12]

The anti-algal action of tannic acid may actually be due to the formation of an oxidized product.[13] Oxidation of phenolics can also produce phytotoxic hydrogen peroxide and superoxide radicals.[14] Ferulate may undergo auto-oxidation under appropriate laboratory conditions (eg, aerobic and alkaline pH)[15] to form hydrogen peroxide, superoxide radicals, and/or a quinone. Pillinger *et al*[13] reported that a class of quinones was at least 1000-fold more anti-algal than ferulate. Ferulate application to catfish ponds may provide catfish producers with an environmentally safe and economical approach to managing musty off-flavor problems in channel catfish production.

In this study, laboratory work was conducted to determine the conditions under which increased ferulate toxicity towards *O* cf *chalybea* might be achieved. Our studies included conducting bioassays to determine the effects of the following factors: (1) light intensity; (2) sodium carbonate peroxyhydrate (SCP) included with ferulate to possibly enhance ferulate oxidation; and (3) a scale-up of the bioassay in which the culture volume in the test was increased from 200 µl to 10 ml (use of six-well microplates). In addition, *cis*-ferulate (Fig 1B), *trans*-methylferulate (Fig 1C), and *trans*-ferulate were tested in a rapid bioassay to determine which form of ferulate is most toxic towards *O* cf *chalybea*. The representative green alga *Selenastrum capricornutum* was also used in each bioassay to determine if toxic selectivity toward *O* cf *chalybea* was maintained as physical and chemical conditions were manipulated.

2 MATERIALS AND METHODS

2.1 Continuous culture conditions

The unialgal culture of *O* cf *chalybea* used in these laboratory studies was previously isolated from the water of a catfish production pond in west-central Mississippi by van der Ploeg *et al.*[16] *S capricornutum* was obtained from Dr JC Greene, United States Environmental Protection Agency (USEPA), Corvallis, OR, and was chosen as a representative for green algae because *Selenastrum* spp are common in freshwater ponds in the southeastern United States.[17] Continuous cultures (approximately 1-litre) of *O* cf *chalybea* and *S capricornutum* were grown and maintained at 29 °C under continuous light by the method of van der Ploeg *et al*,[16] except that medium pH was maintained within a range of 7.6 to 9.0, photosynthetic photon flux density (PPFD), measured with a light meter (LI-COR model LI-250 light meter, LI-COR, Inc, Lincoln, NE), ranged from 18.1 to 28.9 µmol $m^{-2}s^{-1}$, and airflow rate was 16 to 33 litre h^{-1}. Continuous culture samples were measured spectrophotometrically (Gilford model Response UV-VIS spectrophotometer, Gilford Instrument Laboratories, Inc, Oberlin, OH) at 750 nm to monitor cell density. Cell density was maintained at 0.18 to 0.27 A (absorbance) (equivalent to approximately 1.75×10^4 filaments ml^{-1} to 2.65×10^4 filaments ml^{-1} as enumerated using a Sedgwick–Rafter counting chamber) for *O* cf *chalybea* and at 0.19 to 0.26 A (equivalent to approximately 1.92×10^6 cells ml^{-1} to 2.25×10^6 cells ml^{-1}) for *S capricornutum*.

2.2 Effect of light intensity

The bioassay method of Schrader *et al*[18] was adapted to determine if light intensity affected *trans*-ferulate toxicity towards *O* cf *chalybea* and *S capricornutum*. Stock solutions of *trans*-ferulate (99.0% pure; Sigma Chemical Co, St. Louis, MO) were made at 2, 20, 200, 2000, and 20 000 µM in ethanol + water (97.1 + 2.9 by volume) with compensations for purity applied. Ferulate stock solution (10 µl) was placed in the bottom of the wells of the cell culture plate (96-well, polystyrene cell culture cluster, Costar Corporation, Cambridge, MA) and allowed to evaporate completely before 200 µl of either *O* cf *chalybea* culture or *S capricornutum* from continuous cultures were added. Final treatment concentrations were 0.1, 1, 10, 100, and 1000 µM. Controls contained only 200 µl of culture. Four replications were used for each concentration and control. Duplicate plates (two plates per light intensity) were illuminated continuously at PPFDs of 3, 10, 33, and 100 µmol $m^{-2}s^{-1}$ using three overhead fluorescent lamps (40 W, cool white) positioned at various distances from the bench top to provide the desired light intensity. Plates were held at 25–27 °C in a growth chamber. Each experiment was repeated. Absorbance of each well was measured at 650 nm at 24-h intervals for five days using a microplate reader (Bio-Tek model EL311, Bio-Tek

Instruments, Inc, Winooski, VT). Mean values of absorbance measurements for controls and each treatment and their standard deviations were calculated for each experiment and then graphed by plotting mean absorbance readings against time (days). Graphed data (not shown) for each experiment were evaluated individually to determine the Lowest Complete Inhibition Concentration (LCIC) and the Lowest Observed Effect Concentration (LOEC).

2.3 SCP and *trans*-ferulate combinations

O cf *chalybea* and *S capricornutum* were treated with *trans*-ferulate with or without SCP to determine the effects of SCP on the toxicity of *trans*-ferulate. Stock solutions of 4 and 40 μM SCP (77.0% pure; Aldrich Chemical Co, Milwaukee, WI) were prepared in water, filter-sterilized (sterile Acrodisc with 0.2 μm pore size, Gelman Sciences, Ann Arbor, MI), and then added to appropriate treatment wells (50 μl of stock solution per well) containing various concentrations of *trans*-ferulate and either *O* cf *chalybea* or *S capricornutum* continuous culture (150 μl per well). The final treatment concentrations of the SCP–ferulate combinations were 1 and 10 μM SCP with 0.1, 1, 10, and 100 μM ferulate. Previous screening studies using *O* cf *chalybea* had demonstrated that the LCIC and LOEC for SCP were 100 μM and 1 μM, respectively,[19] and the LCIC and LOEC for ferulate were 1000 μM and 1 μM, respectively.[4] Using *S capricornutum*, the LCIC and LOEC for ferulate and for SCP were 1000 μM.[4,19] The final concentrations of SCP and ferulate used in this study are below the LCIC for each compound to help determine if the combination of SCP and ferulate is synergistically toxic towards *O* cf *chalybea*.

Controls contained 150 μl of culture and 50 μl of filter-sterilized deionized water. Plates were held at 29 °C under continuous light at a PPFD of 21 to 27 μmol $m^{-2}s^{-1}$. Four replications were used for each treatment of SCP with ferulate and for controls, duplicate plates were used for each experiment, and the experiment was repeated. Data from each experiment were graphed and evaluated individually to determine LCIC and LOEC.

2.4 Effect of increased culture volume used in bioassay

A scale-up of the bioassay to six-well microplates was performed. Six-well microplates (polystyrene cell culture cluster, Costar Corporation, Cambridge, MA) were used in the following manner: (1) one well was used as a blank and contained only sterile deionized water; (2) two wells were used as controls and contained only 10 ml of either *O* cf *chalybea* or *S capricornutum* culture; and (3) the remaining three wells were used as treatments and contained *trans*-ferulate (one concentration per plate and three replications per concentration) and either *O* cf *chalybea* or *S capricornutum* culture. Ferulate stock solutions in ethanol (20, 200, 2000, and 20 000 μM) were micropipetted into treatment microplate wells (0.5 ml per well) and the ethanol was allowed to evaporate completely before adding 10 ml of either *O* cf *chalybea* or *S capricornutum* culture. Final ferulate concentrations tested were 1, 10, 100, and 1000 μM. Plates were placed in a growth chamber at 25–27 °C and were illuminated continuously at a PPFD of 21–27 μmol $m^{-2}s^{-1}$. Absorbances of each well were measured at 650 nm at 24-h intervals for six days using a microplate reader (Packard model SpectraCount microplate photometer, Packard Instrument Co, Meriden, CT) which was modified in our laboratory to take 182 readings per well (for a six-well plate) using modified software (version 2.10, Build 28 Beta) provided by Packard Instrument Company. This modification permitted absorbance measurements of the entire well surface and yielded reproducible results when performing six-well microplate bioassays with *O* cf *chalybea* which has a tendency to clump in the six-well microplate wells. The experiment was repeated, and the LCIC and LOEC values were determined from graphed data of the six-well microplate bioassay and compared to LCIC and LOEC values obtained from a 96-well microplate bioassay run concurrently by the method of Schrader *et al.*[18]

2.5 Toxicity of chemically and structurally modified forms of ferulate

trans-Methylferulate [methyl 3-(4-hydroxy-3-methoxyphenyl)-2-propenoate], *cis*-ferulate, and *trans*-ferulate were screened to determine the form of ferulate that is most toxic towards *O* cf *chalybea*. *Cis*-ferulate was prepared by UV irradiation (λ 254 nm) of a methanol solution (1 mg ml^{-1}) of *trans*-ferulate over a 24-h period. The conversion of *trans*- to *cis*-ferulate was monitored by GC/MS analysis of their trimethylsilyl (TMS) derivatives using a DB-5 capillary column (30 m length × 0.32 m internal diameter × 25 μm film thickness) and a temperature program as follows: initial temperature of 120 °C held for 1 min, ramped 6 °C min^{-1} to 325 °C and held at this temperature for 5 min; injector port temperature of 250 °C; transfer line temperature of 280 °C; helium flow rate of 1.81 ml min^{-1} (RT *cis*-ferulic-TMS 13.70 min, *trans*-ferulic-TMS 16.57 min). *Cis*-ferulate was purified by HPLC using a μBondapak™ preparative C18 column (19 mm × 300 mm) eluted with acetonitrile/water (gradient 0% to 30% CH_3CN at 0–20 min, 30% to 100% CH_3CN at 20–25 min, 100% CH_3CN at 25–35 min) and a flow rate of 4 ml min^{-1} (RT *cis*-ferulate 15.70 min, *trans*-ferulate 17.04 min monitored at λ 254 nm).

Methylation of a 1 mg ml^{-1} methanol solution of *trans*-ferulate was performed by adding an excess of diazomethane. The reaction was allowed to stand for 4 h at room temperature. The product was purified by thin layer chromatography using chloroform + methanol (95 + 5 by volume) as developing solvent and was identified from its [^{1}H]NMR and mass spectrum data as methyl 3-(4-hydroxy-3-methoxyphenyl)-2-propenoate. [^{1}H]NMR in deuterochloroform (δ ppm,

multiplicity, $\mathcal{J}$ in Hz; see Fig 1C for numbering): 7.55, d, 15.8, 3-H; 7.00, d, 8.3, 9-H; 6.95, s, 5-H; 6.84, d, 8.3, 8-H; 6.22, d, 15.8, 2-H; 5.86, s, 7-OH; 3.84 and 3.72 each s, 10-OCH_3 and 11-OCH_3, interchangeable. EIMS data (70 eV): *m/z* 208 M^+; 193 (M^+—CH_3), 177 (M^+—OCH_3), 145 ([177]$^+$—OCH_3—H).

Stock solutions of *trans*-methylferulate (100.0% pure) were made at 20, 200, 2000, and 20 000 μM in ethanol + water (97.1 + 2.9 by volume). Final treatment concentrations were 1, 10, 100, and 1000 μM. *trans*-Methylferulate screening results were compared to *trans*-ferulate screening results, also run at final treatment concentrations of 1, 10, 100, and 1000 μM. For comparison of toxicity between *cis*- and *trans*-ferulate, stock solutions of *cis*-ferulate (100.0% pure) and *trans*-ferulate (99.0% pure) were made at 0.1, 1, and 10 mM due to the limited quantity of *cis*-ferulate that was available. Final treatment concentrations were 5, 50, and 500 μM. Microplate bioassays were set up and analyzed in the same manner as described previously for the study of variation of light intensity, except that plates were illuminated continuously at a PPFD of 21–27 μmol $m^{-2}s^{-1}$, three replications were used for each concentration and control, and absorbances were measured at 24-h intervals for four days.

3 RESULTS AND DISCUSSION

The mode of action of ferulate phytotoxicity is not at present known. However, photo-oxidation of phenolic compounds can result in the formation of singlet oxygen ($^1O_2$).[20] Singlet oxygen and other photo-oxidants produced in sunlight can further enhance the photo-oxidation of phenolics.[21] In aquatic ecosystems, dissolved natural organic material strongly promotes the phototransformation of phenolic compounds.[21,22] Therefore, *trans*-ferulate applied to aquaculture ponds may undergo sensitized photo-oxidation to a variety of phytotoxicants. Increasing light intensity may increase the photo-oxidation of phenolics, as well as the formation of phytotoxic hydrogen peroxide, singlet oxygen, superoxide radicals, and/or a quinone. Based on the derived LOEC values, increasing light intensity enhanced the toxicity of *trans*-ferulate towards *O* cf *chalybea* (Table 1) but not towards *S capricornutum* data not shown. The LCIC values were unaffected by light intensity. These results indicate that the mechanisms of action of ferulate phytotoxicity on *O* cf *chalybea* and *S capricornutum* may differ. Increased ferulate oxidation may be occurring as light intensity increases. If ferulate does undergo photo-oxidation in catfish ponds to yield more strongly phytotoxic compounds such as certain quinones, the phototransformation process may occur gradually, so that the removal of cyanobacterial blooms would create less of a 'shock' to the ecosystem, and, thereby, help to prevent such problems as low dissolved oxygen and subsequent fish kills associated with rapid algal bloom die-offs. Further studies on the effects of light quality and quantity may provide additional information on the potential effects on the efficacy of ferulate in field situations.

Table 1. Effect of light intensity on *trans*-ferulate toxicity towards *Oscillatoria* cf *chalybea*

Photosynthetic photon flux density (μmol $m^{-2}s^{-1}$)	*LCIC*[a] *(μM)*	*LOEC*[b] *(μM)*
3.0	1000	10
10.0	1000	100
33.0	1000	1
100.0	1000	0.1

[a] Lowest-complete-inhibition concentration.
[b] Lowest-observed-effect concentration.

SCP is a strong oxidizing compound, and therefore the addition of SCP to media containing ferulate might enhance its oxidation. The growth of *O* cf *chalybea* has been reported to be affected (inhibited, but not completely) by concentrations of ferulate and SCP as low as 1 μM.[4,19] SCP is more environmentally acceptable than synthetic compounds (herbicides) for use in aquaculture [SCP forms hydrogen peroxide and sodium carbonate upon contact with water], and, therefore, SCP could be used safely with ferulate to help control problematic cyanobacterial blooms.

The combination of SCP and *trans*-ferulate at sublethal concentrations did not enhance their toxicity towards either *O* cf *chalybea* (Table 2) or *S capricornutum* (data not shown). In fact, combinations of 1 μM or 10 μM SCP with 1 μM *trans*-ferulate were less toxic towards *O* cf *chalybea* than 1 μM SCP or *trans*-ferulate alone.[4] These results may be due to a quenching of the toxicity of superoxide anion radicals (O_2^-) by their combination with hydrogen peroxide formed from SCP. A reduction in the quantity of O_2^- may allow the antioxidant enzyme systems(s) of *O* cf *chalybea* to scavenge the oxygen radicals, whereby previously the capacity of these systems had been exceeded.[14] In addition, sodium carbonate may combine with carbon dioxide and water to form sodium bicarbonate, which causes a pH increase. Low carbon dioxide levels have

Table 2. Effect of concentration of SCP on *trans*-ferulate toxicity towards *Oscillatoria* cf *chalybea*

Treatment		*Effect on the growth of O cf chalybea*[b]
trans-Ferulate (μM) and SCP[a] *(μM)*		
0.1	1	No effect
1	1	No effect
10	1	Inhibitory[c]
100	1	Inhibitory[c]
0.1	10	No effect
1	10	No effect
10	10	Inhibitory[c]
100	10	Inhibitory[c]

[a] Sodium carbonate peroxyhydrate.
[b] Determined from graphed data.
[c] Growth inhibition was not complete.

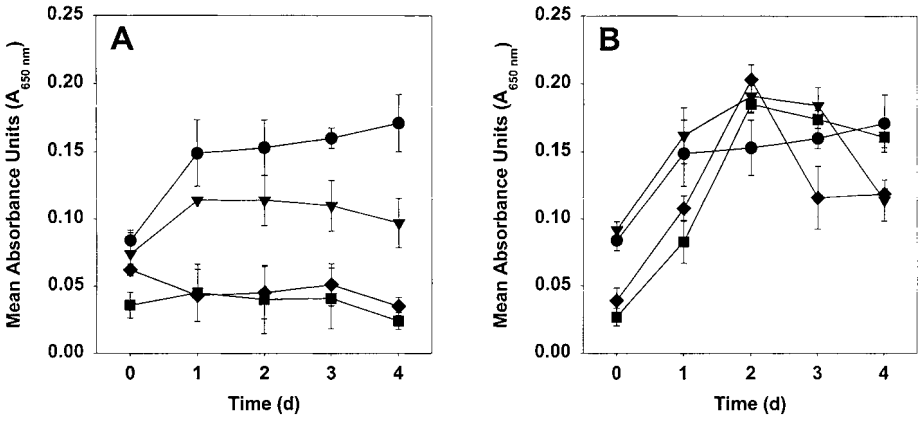

Figure 2. Effect of concentrations (5, 50, and 500 μM) of (A) *trans*-ferulate and (B) *cis*-ferulate on the growth of *Oscillatoria* cf *chalybea* ($n = 3$). (●) Control; (▼) 5 μM; (◆) 50 μM; (■) 500 μM. The vertical bars represent the standard error.

been reported to enhance the activity of the protective enzyme system of the cyanobacterium *Nostoc muscorum* for hydrogen peroxide removal.[23] Alkaline pH should increase the auto-oxidation potential of phenolic compounds; however, the presence of non-enzymatic oxidants may play a greater role in the oxidation of phenolics. Also, certain metabolites produced by microbes may act as oxidants of phenolics,[14] and an increase in pH might affect the production of such metabolites by *O* cf *chalybea*.

Trans-Ferulate toxicity towards *O* cf *chalybea* was the same in the six-well microplate bioassay as in the 96-well microplate bioassay based upon LCIC results (1000 μM). However, *trans*-ferulate was less toxic towards *O* cf *chalybea* in the six-well microplate bioassay than in the 96-well microplate bioassay based upon LOEC results of 10 μM and 1 μM, respectively. *Trans*-Ferulate toxicity towards *S capricornutum* in the six-well microplate bioassay was the same as in the 96-well microplate bioassay with LCIC and LOEC values of 1000 μM. Growth conditions in the wells of six-well and 96-well microplates may influence the concentrations of carbon dioxide and oxygen in the culture of *O* cf *chalybea* placed in these wells, and *O* cf *chalybea* may be more sensitive to these conditions than *S capricornutum*. In our study, the surface-area-to-culture volume (S:V) used in the wells of the 96-well microplate was 10 times greater than the S:V used for the wells of the six-well microplate. The gas exchange between the atmosphere and liquid culture in the six-well microplate wells would be reduced compared to the gas exchange in the 96-well microplate wells. Because photosynthetic activity occurs during the bioassay, oxygen would increase and carbon dioxide levels would decrease in cultures placed in six-well microplate wells more than in cultures placed in the 96-well microplate.

If *trans*-ferulate is undergoing auto-oxidation in the microplate wells, hydrogen peroxide and O_2^- produced from the reaction may be removed by the anti-oxidative enzyme systems of *O* cf *chalybea*. The anti-oxidative enzyme systems usually present in cyanobacteria to remove hydrogen peroxide and O_2^- produced during photosynthetic activity are catalase and superoxide dismutase (SOD), respectively.[24] In catalase-lacking species of cyanobacteria, ascorbate peroxidase removes low concentrations of hydrogen peroxide.[25] Currently, the protective enzyme systems used by *O* cf *chalybea* to detoxify hydrogen peroxide and O_2^- are unknown; however, SOD is present in cyanobacteria irrespective of the enzyme system that they might use for protection from O_2^-.[25] An increase of SOD production in bacteria exposed to increasing concentrations of atmospheric O_2 has been demonstrated.[26] Tel-Or *et al*[23] reported that ascorbate peroxidase activity in the cyanobacterium *Synechococcus* 6311 and catalase activity in *N muscorum* were directly related to atmospheric carbon dioxide levels. Additional amounts of hydrogen peroxide and O_2^- produced during ferulate oxidation may overwhelm the capacity of the protection mechanisms in *O* cf *chalybea* if such mechanisms are unable to respond as efficiently under conditions that suppress antioxidant enzyme activity, ie, reduced oxygen levels and increased carbon dioxide levels.

The *trans*- form of ferulate when tested on *O* cf *chalybea* was more toxic than *cis*-ferulate (Figs 2A and 2B) and *trans*-methylferulate (Figs 3A and 3B). Also, the *trans*-form of ferulate was more toxic towards *S capricornutum* than *cis*-ferulate (data not shown); however, *trans*-methylferulate was slightly more toxic towards *S capricornutum* than *trans*-ferulate (Figs 4A and 4B). The three concentrations of *cis*-ferulate screened (5, 50, and 500 μM) were not inhibitory towards *O* cf *chalybea* until two to three days while 5, 50, and 500 μM of *trans*-ferulate were inhibitory at one to two days *cis*-Ferulate at 500 μM (Fig 2B) and *trans*-methylferulate at 1000 μM (Fig 3B) were not completely inhibitory towards *O* cf *chalybea* until two to three days while *trans*-ferulate at 500 μM and 1000 μM was completely inhibitory within 24 h (Figs 2A and 3A, respectively). In addition, 1 and 10 μM of *trans*-ferulate were inhibitory after 24 h towards *O* cf *chalybea*, while 1 and 10 μM of *trans*-methylferulate were not toxic towards *O* cf *chalybea* until three to four days *O* cf *chalybea* culture added to microplate wells containing 100 and 1000 μM of *trans*-methylferulate turned yellow after 24 h. This color change did not occur in any of the other microplate wells containing concentrations below 100 μM of *trans*-methylferulate or in any of the

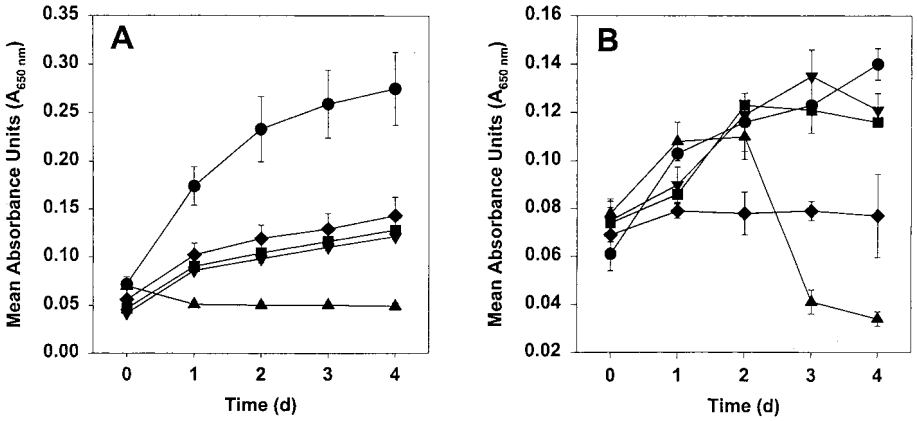

Figure 3. Effect of concentrations (1, 10, 100, and 1000 μM) of (A) *trans*-ferulate and (B) *trans*-methylferulate on the growth of *Oscillatoria* cf *chalybea* ($n=3$). (●) Control; (▼) 1 μM; (■) 10 μM; (◆) 100 μM; (▲) 1000 μM. The vertical bars represent the standard error.

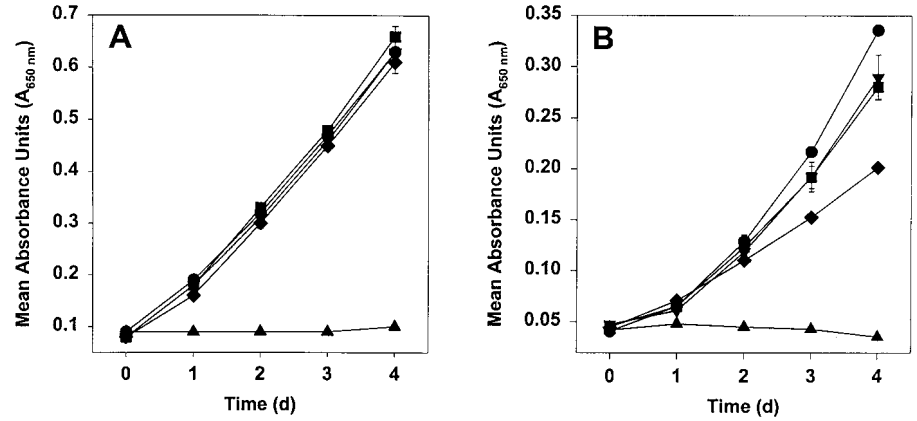

Figure 4. Effect of concentrations (1, 10, 100, and 1000 μM) of (A) *trans*-ferulate and (B) *trans*-methylferulate on the growth of *Selenastrum capricornutum* ($n=3$). (●) Control; (▼) 1 μM; (■) 10 μM; (◆) 100 μM; (▲) 1000 μM. The vertical bars represent the standard error.

microplate wells containing either *trans*-ferulate or *cis*-ferulate. Phenolic compounds can undergo oxidation that leads to the formation of colored compounds.[27] One condition that regulates the oxidation of phenolic compounds is phenolic structure,[14] and this factor might account for the difference in toxicities of *trans*-ferulate, *cis*-ferulate, and *trans*-methylferulate towards *O* cf *chalybea*. Apparently, the increased potential for the cellular transport of *trans*-methylferulate into cells of *O* cf *chalybea* had little effect on enhancing ferulate toxicity towards *O* cf *chalybea*.

4 CONCLUSIONS

Laboratory studies were conducted to determine the effects of oxidants and light intensity on ferulate toxicity towards *O* cf *chalybea*, as well as the phytotoxic effects of ferulate analogs on *O* cf *chalybea*. The results from these studies were evaluated to help determine methods for increasing the cost-effectiveness of using *trans*-ferulate as a selective algicide and to determine the conditions and modifications which should be used in conducting field-trial studies of *trans*-ferulate. These studies revealed the following: (1) sunlight may have an effect on the stability and efficacy of ferulate applied to fish ponds to control *O* cf *chalybea;* (2) the inclusion of an oxidizing compound with ferulate applications to fish ponds would probably not enhance ferulate toxicity towards *O* cf *chalybea* in fish ponds; (3) *trans*-ferulate is more toxic towards *O* cf *chalybea* than *cis*-ferulate and *trans*-methylferulate, and therefore, *trans*-ferulate should be used in any field trials; and (4) the reduction of *trans*-ferulate toxicity towards *O* cf *chalybea* in the progression from a 96-well microplate well (200 μl) to a larger-volume system indicates the need for using higher, less cost-effective concentrations of *trans*-ferulate for controlling *O* cf *chalybea* in fish ponds. Field trials will ultimately reveal the actual persistence and toxicity of *trans*-ferulate towards *O* cf *chalybea* in aquaculture ponds and will help to determine if *trans*-ferulate is worthwhile pursuing as a lead compound for the selective control of noxious cyanobacteria in fish ponds.

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